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The genetics of *Artemia salina*

VII. Reproductive isolation

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THE BRINE SHRIMP lives in seacoast salterns and inland salt lakes on six continents. Each clone of parthenogenetic shrimps is reproductively isolated. Among gonochoristic populations, some are reproductively isolated from the others[5,11,12,14]. This paper summarizes reproductive isolation among 27 populations. Ten of the 21 gonochoristic populations have not been described previously.

Materials and Methods

Culture techniques. Cysts (encysted blastulae) collected in the natural habitat were hatched in artificial sea water. The first instar nauplius was immediately transferred to culture medium D that contained in one liter: 1 g sodium tetraborate, 50 g NaCl, and 40 g of solid component of "Instant Ocean" (Synthetic Sea Salts, Aquarium Systems, Inc., Wickliffe, Ohio). This solution was boiled, cooled and filtered to remove the precipitate that appeared upon boiling. All cultures were maintained in 5 ml of medium D in shell vials (21 mm diameter, 70 mm high). Two nauplii were placed in each vial. Every seventh day, each culture received 0.05 ml of MMM yeast suspension. (MMM is made by combining one 17 g cake of living bakers yeast (Standard Brands, Inc.), 4 g dry brewers yeast (Nutritional Biochemicals Co.) and 90 ml of culture medium D.) Cultures were maintained in the dark at 23°C except for the few hours each week when they were examined and fed. This culture method, designed for optimum viability and fast maturation, gave viability values of 80 percent for San Francisco shrimps at 2 weeks of age, at which time they were sexually mature (vitellogenesis visible in most females). Viability was lower in shrimps from Hidalgo, San Bartolomeo, and Tunis and in the F_1 progeny of San Bartolomeo × Tunis. The San Bartolomeo, Urmia, and parthenogenetic females required more time to mature (3–5 weeks).

Matings. To ensure nonimpregnation of females, they were isolated from males for a period of 2 weeks or more. The reproductive cycle was completed within one week and genetic experiments have shown that females do not store sperm from one cycle to the next. Shrimps were transferred from one vial to the next in glass pipets. These were placed in boiling water for more than 2 minutes before being used to pick up shrimps of different genotype.

All matings were single-pair matings. They were checked every 7 days and nauplii, if present, were transferred to other vials (2 nauplii per vial). If one member of a mated pair died without progeny within a 2-week period, data from that mating were discarded. Matings were maintained for at least 2 weeks before they were designated infertile. Data were collected on the frequency of clasping pairs. In those crosses found to be infertile (e.g., TUN × URM), the males clasped the females as frequently as in the fertile crosses.

Origin of gonochoristic populations. Cysts were collected from: GSL, Great Salt Lake, Utah; ING, Inagua, salterns on Greater Inagua Island, West Indies; HDL, Hidalgo, Salina Grande, La Pampa, Argentina; JMS, James, natural lagoon on James Bay, James Island (Isla Santiago), Galapagos Islands; KGR, Kiatuthlanna Green Pond, 30 km northwest of St. Johns in Apache County, Arizona, on the Long H Ranch, 60 km southwest of Zuni, New Mexico[8,9]; KRD, Kiatuthlanna Red Pond, 0.4 km from KGR; LMN, Little Manitou Lake, Saskatchewan, Canada (150 km north of Moose Jaw and 50 km west of Big Quill Lake[18]); MNO, Mono Lake, Mono County, California[16]; MSS, salterns 0.3 km north of Elkhorn Slough near Moss Landing on Monterey Bay, California; PCH, salterns at Pichilingue Island, in the Gulf of California, near La Paz, Mexico; QMD, salt lake 30 km northwest of Quemado in Catron County, New Mexico[6]; RCK, salterns at Rockhampton, at the mouth of the Fitzroy River, Queensland, Australia; SBT, salterns at San Bartolomeo, Cagliari, Sardinia; SDG, salterns at San Diego, California; SPL, Soap Lake, eight miles south of Okanogan, Washington; SQN, natural lagoon at San Quintin, on the west coast of Baja California, Mexico; TLL, salterns of Ponce Salt Industries on Bahia de Tallaboa, Puerto Rico; TUN, Chott Ariana at Raoued, Tunisia; URM, Lake Urmia (Lake Rezaiyeh), Iran; VLW, Vallejo West Pond, 3.8 km east of Sonoma Creek and 7 km west of the Napa River, near Vallejo, California; SFR, saltern A18 of the Leslie Salt Company on the southernmost point of San Francisco Bay, California. This 850 acre pond was the source of most commercial eggs packaged by San Francisco Bay Brands Company in the years 1971–

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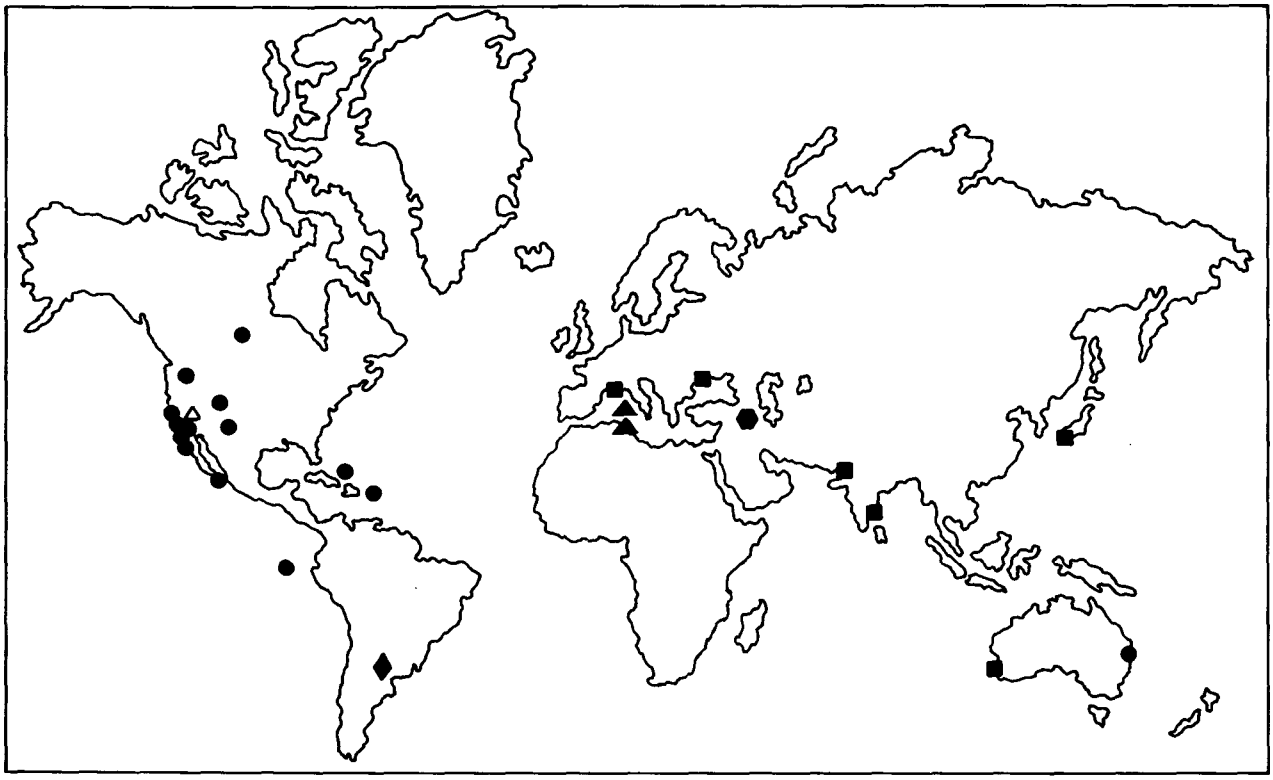

FIGURE 1—Speciation in *Artemia*. Squares represent the six parthenogenetic populations. Other symbols represent gonochoristic populations: circles, the 16 populations designated as CRSFR (San Francisco and those crossfertile with San Francisco); open triangle, Mono Lake; solid triangles, San Bartolomeo and Tunis; diamond, Hidalgo; hexagon, Lake Urmia. One circle denotes three adjacent populations (KGR, KRD, QMD).

1972. (Personal communication from Irving Fishman, Technical Director, San Francisco Bay Brand, Inc.) Specific gravity ranged from 1.07 to 1.10. A limnological study of adjacent salterns was made by Carpelan[7].

Origin of parthenogenetic populations. Cysts from thelytokous parthenogenetic females were collected from Madras and from the Gulf of Kutch, India; Odessa, Russia; Rottnest, Australia; Sète, France; and Yamaguchi-ken, Japan. The distribution of populations is shown in Figure 1.

Origin of inbred stocks. Line #9 carries the gene *w*, a recessive partially sex-linked gene for white eyes, described previously[5]. Stock #51 is homozygous for *w* and has a genetic background derived from SFR, GSL, and other populations. Stock #52 is derived only from SFR shrimps and is homozygous for an allele of *w*, chalky (w^{ch}). In this paper, the white-eyed phenotype may be due either to *w*/*w* or w^{ch}/w^{ch} genotype.

CRSFR. This acronym for "cross fertile with San Francisco (SFR) *Artemia*", denotes San Francisco shrimps, the 15 populations crossfertile with them, and the inbred stocks derived from any of these 16 wild populations. Operationally, we identify "population A" as CRSFR by making single-pair matings and determining the ratio of fertile matings to total matings. This ratio is determined for intrapopulation matings (A × A) and for reciprocal outcrosses (A × SFR). If population A is CRSFR, the outcross ratio is at least 20 percent as great as the value for intrapopulation crosses, the F_1 progeny are fertile and normal segregation of a mutant gene must be observed in the F_2.

Results

Five gonochoristic populations are not crossfertile with San Francisco shrimps. The habitat isolation of Mono Lake shrimps was discussed earlier[4]. Hidalgo *Artemia* appear to be reproductively isolated from San Francisco, Tunis, and San Bartolomeo shrimps (Table I). Although Tunis and San Bartolomeo shrimps are crossfertile with one another, they are reproductively isolated from San Francisco (Table II) and Lake Urmia shrimps (Table III). Lake Urmia shrimps are not crossfertile with San Francisco. Intrapopulation matings were made to obtain control data on fertility and were observed for only a 2-week period, whereas experimental crosses were observed as long as both mates lived. In Tables I and III, the number of weeks of observation is given (sum of the products of number of matings × weeks each was observed).

Of the 21 gonochoristic populations, 15 are crossfertile with San Francisco shrimps: GSL, ING, JMS, KGR, KRD, LMN, MSS, PCH, QMD, RCK, SDG, SPL, SQN, TLL, and VLW. Shrimps hatched from cysts collected in the natural habitat were mated to CRSFR shrimps, some of which were homozygous for the recessive mutation for white eyes. From reciprocal crosses, fertile F_1 and normal F_2 progeny were obtained.

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The expected ratios of normal to mutant phenotype were observed in the F_2 from each racial cross.

To support the concept that the populations listed in Table IV are not reproductively isolated from San Francisco shrimps, data were collected to exclude the possibility of hybrid breakdown. Females from each of six populations were hatched from cysts and mated to white-eyed males from stock #51. Because the female shrimp is heterogametic (XY) and *w*, the gene for white eyes, is partially sex-linked (located on the homologous portion of the sex chromosomes)[5], these females may be designated as Y_+X_+ and the males as $X_w{}^{51}X_w{}^{51}$. The F_1 females, $Y_+X_w{}^{51}$, were testcrossed to stock #51 males. All matings listed in Table IV yielded progeny that fit the expected pattern of four classes of testcross progeny: two large classes of nonrecombinants (black-eyed females and white-eyed males) and two small classes of recombinants (white-eyed females and black-eyed males).

The frequency of crossing over between the white locus and the sex locus is determined by the source of the differential segment of the Y chromosome[5]. Crossover frequency can approach zero when a certain Y chromosome is present (the Y from stock #9). In the testcrosses described above, crossover frequency ranged from 0.9 to 18 percent. These values do not exceed the range found in the San Francisco population[5].

Table I. Evidence that the Hidalgo (HDL) population is reproductively isolated from the Tunis (TUN) and San Bartolomeo (SBT) populations and also from those shrimps "crossfertile with San Francisco" (CRSFR); each wild-type shrimp hatched from a cyst collected in the natural habitat

Parental cross ♀ × ♂	Parental crosses: fertile matings/ total matings	Total no. weeks matings observed†
HDL × HDL (control)	19/28	56
HDL × TUN	0/8	20
TUN × HDL	0/17	51
HDL × SBT	0/14	32
SBT × HDL	0/4	12
SFR × HDL	0/19	42
HDL × CRSFR*	0/23	83
CRSFR × HDL	0/29	76

* In this cross, CRSFR shrimps were white-eyed shrimps from inbred stocks #51 or #52

† Sum of products of no. matings × weeks each observed

Table II. Evidence that the Tunis (TUN) and San Bartolomeo (SBT) populations are crossfertile with one another but are reproductively isolated from other gonochoristic populations; each TUN and SBT shrimp was hatched from a different cyst collected from the natural habitat

Parental cross ♀ × ♂	Parental crosses: fertile matings/ total matings	Adult F_2 progeny from each fertile parental pair
TUN × TUN (control)	21/33	Yes
SBT × SBT (control)	15/24	Yes
TUN × SBT	9/13	Yes
SBT × TUN	8/10	Yes
TUN × CRSFR*	0/21	—
CRSFR × TUN	0/24	—
SBT × CRSFR*	0/4	—
CRSFR × SBT	0/15	—

* CRSFR shrimps are cross fertile with San Francisco (SFR) population; in this cross, CRSFR shrimps were white-eyed (stock #51) or were hatched from cysts from Great Salt Lake

Table III. Evidence that shrimps from Lake Urmia are reproductively isolated from those from Tunisia and from shrimps crossfertile with San Francisco (stock #51); each wild-type shrimp hatched from different cyst collected in natural habitat

Parental cross ♀ × ♂	Parental crosses: fertile matings/ total matings	Total no. weeks matings observed*
URMIA × URMIA (control)	6/15	30
URMIA × TUNIS	0/18	64
TUNIS × URMIA	0/25	85
URMIA × SFR	0/16	72
URMIA × #51	0/26	93
#51 × URMIA	0/31	106

* Sum of products of no. matings × weeks each observed

Table IV. Crossing over in F_1 females derived from matings of white-eyed males with females from six wild populations; crossover frequency determined by origin of female parent (and of Y chromosome)

Origin of parents ♀ × ♂	Genotype of F_1 ♀♀*	Progeny of F_1 ♀♀ × *w*/*w* ♂♂ (recombinants/ total progeny)	
cyst #1, ING × #51	$Y_+{}^{ING1}X_w{}^{51}$	8/94	9%
cyst #2, ING × #51	$Y_+{}^{ING2}X_w{}^{51}$	23/214	11
cyst #3, ING × #51	$Y_+{}^{ING3}X_w{}^{51}$	21/125	17
cyst #1, JMS × #51	$Y_+{}^{JMS1}X_w{}^{51}$	44/323	14
cyst #1, RCK × #51	$Y_+{}^{RCK1}X_w{}^{51}$	9/215	4
cyst #2, RCK × #51	$Y_+{}^{RCK2}X_w{}^{51}$	17/168	10
cyst #3, RCK × #51	$Y_+{}^{RCK3}X_w{}^{51}$	26/145	18
cyst #1, SQN × #51	$Y_+{}^{SQN1}X_w{}^{51}$	21/304	7
cyst #2, SQN × #51	$Y_+{}^{SQN2}X_w{}^{51}$	25/176	14
cyst #3, SQN × #51	$Y_+{}^{SQN3}X_w{}^{51}$	30/209	14
cyst #5,† SFR × #51	$Y_+{}^{SFR5}X_w{}^{51}$	7/820	0.9
cyst #1, TLL × #51	$Y_+{}^{TLL1}X_w{}^{51}$	18/205	9
cyst #2, TLL × #51	$Y_+{}^{TLL2}X_w{}^{51}$	17/168	10
cyst #3, TLL × #51	$Y_+{}^{TLL3}X_w{}^{51}$	58/420	14
cyst #4, TLL × #51	$Y_+{}^{TLL4}X_w{}^{51}$	39/241	16
#51 × SFR	$Y_w{}^{51}X_+{}^{SFR}$	12/101	12
#9 × #51	$Y_+{}^{9}X_w{}^{51}$	0/135	0
#9 × #9	$Y_+{}^{9}X_w{}^{9}$	1/5068	0.02

* Subscripts indicate allele at white locus; superscripts, origin of differential segment of Y chromosome

† Recombination frequencies of four other Y chromosomes from SFR females and from females of other populations were published earlier

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Discussion

San Francisco *Artemia* are diploid ($2n = 42$)[2,12]. Because we found 15 populations to be crossfertile with San Francisco shrimps, we conclude that they are also diploid. An earlier study in our laboratory[5] showed one successful mating of an Hidalgo male with shrimps crossfertile with San Francisco. Our recent attempts to hybridize Hidalgo with either San Francisco or Tunis shrimps failed (Table I). This is in accord with the report[12] that the Hidalgo population may be a mixture of sibling species.

We conclude that the San Bartolomeo and Tunis populations are crossfertile. Both are reproductively isolated from California shrimps, confirming earlier reports[5,12,14] for San Bartolomeo *Artemia* and in accord with Gilchrist[11] for "Algerian" shrimps.

Barigozzi[2] has proposed that the San Francisco population be designated *A. franciscana* and the biotypes predominating at San Bartolomeo and Hidalgo[12] be called *A. salina* and *A. persimilis*, respectively. We agree that the gonochoristic populations include sibling species and incipient species. However, the term *A. salina* has been used in the past for all populations and confusion will result if it is now restricted to the San Bartolomeo population. We suggest that this term be dropped entirely rather than changed in meaning. We will not assign species names to the Mono and Urmia populations until we have characterized them more completely in regard to biochemical traits. We will determine if they are mixtures of sibling species as appears to be the case for the Hidalgo shrimps[12].

The widespread distribution of 16 cross-fertile gonochoristic populations (Figure 1) suggests that this genotype must have been disseminated by birds or commercial introduction. Cysts (encysted blastulae) can be transported by birds, either on the feathers or in the digestive tract. Viable cysts have been recovered from the feces of *Anas platyrynchos*[17]. Anderson has listed 55 species of birds seen at the San Francisco salterns, including *A. platyrynchos*, *Larus californicus*, *Numenius phaeopus*, *Steganopus tricolor*, *Podiceps caspicus*, and *Recurvirostra americana*. In the latter three species, he reported that brine shrimps made up a substantial portion of the diet[1]. The California gull (*Larus*) occurs on the coast from Alaska to Mexico and inland in southern Canada, North Dakota, and Great Salt Lake. Gulls banded at Mono Lake make a fall east-west migration to San Francisco and Monterey Bay[11]. The whimbrel (*Numenius*) and Wilson's phalarope (*Steganopus*) winter in southern South America and have been sighted in the Galapagos[15]. Dispersal of the North African biotype to San Bartolomeo (or the reverse) may have been by means of bird migrations. For example, the gull *Larus ridibundus* migrates between Europe and Tunisia by way of Sardinia[10].

A second mode of dispersal is man's intentional introduction of *Artemia* into salterns. Where salt is produced commercially by solar evaporation, *Artemia* is introduced in the belief this will reduce the amount of algae in the brine. Although the *Artemia* population at Rockhampton, Australia, has flourished for at least 15 years, the workmen have stated that shrimps were introduced from a packet of commercial eggs (personal communication from Mr. M.V. Melvin, Senior Production Officer, ICI Australia Petrochemicals Ltd.). If this were the case, the most likely source would have been Great Salt Lake or San Francisco Bay. Mr. Maurice Rakowicz reported that in 1971 he introduced San Francisco cysts into natural lagoons at Christmas Island (1600 km south of Hawaii) to initiate an aquaculture project.

Summary

Fifteen of 20 gonochoristic *Artemia* populations are crossfertile with diploid San Francisco shrimps, producing fertile F_1 and viable F_2 progeny. Partial sex linkage of white eye was observed and frequency of crossing over between the white and sex loci did not exceed the range of values observed in San Francisco shrimps. Possible mechanisms for wide dispersal of this diploid genotype are discussed.

Five populations are reproductively isolated from San Francisco shrimps: Mono Lake, Hidalgo, Lake Urmia, San Bartolomeo, and Tunisia. The last two are interfertile.

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